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Eurasian Watermilfoil Control Using Contact Herbicide Phenological Timing

PURPOSE: This technical note evaluates the efficacy of the contact herbicide Aquathol-K upon the exotic weed Eurasian watermilfoil (*Myriophyllum spicatum* L.) when applications are timed to coincide with periods of low carbohydrate storage within the target plant. This herbicide demonstration study was based on previous phenological research to determine when Eurasian watermilfoil has the least amount of stored carbohydrates available for regrowth (Madsen 1997b). By timing the herbicide application with low total nonstructural carbohydrate storage, aquatic plant managers can maximize the effectiveness of the herbicide treatment.

BACKGROUND: Eurasian watermilfoil, as implied by the common name, is native to Europe and Asia and was first discovered in the United States in 1942 near Washington, DC (Couch and Nelson 1985). It has since spread to 43 states (Florida Caribbean Science Center 1998).

Eurasian watermilfoil exhibits an aggressive growth strategy, rapidly elongating through the water column and forming a dense surface canopy (Madsen 1997b). This dense surface canopy can impede navigation, degrade water chemistry and native habitat, and interfere with recreational and fisheries usage (Madsen 1997a). Although Eurasian watermilfoil can reproduce by seed, the most effective method of reproduction is by stolons and vegetative production of autofragments (Madsen and Smith 1997; Madsen, Eichler, and Boylen 1988).

Standard techniques currently available for managing Eurasian watermilfoil include mechanical, physical, biological, and chemical methods. Chemical techniques utilize U.S. Environmental Protection Agency-registered aquatic herbicides that have different mechanisms of action and product-specific application rates. Aquathol-K is a formulation of endothall, a nonselective contact herbicide that inhibits protein synthesis and limits translocation throughout the plant tissue. This herbicide provides excellent control of Eurasian watermilfoil in ponds and whole-lake systems (Westerdahl and Getsinger 1988).

Phenological studies of Eurasian watermilfoil provide information that can be used to maximize the efficiency of control techniques. At the beginning of the growing season, stored total nonstructural carbohydrates (TNC) are at high levels in the storage organs (lower shoots and root crowns, Figure 1a). The TNC are used by the new spring growth as they are translocated to the upper shoots (Figures 1a, 1b).

At a certain point in the growth cycle, plant production of TNC exceeds plant requirements, and the excess carbohydrates are exported to the storage organs (Figures 1a, 1b). Just prior to this exportation to the storage organs, carbohydrates within the storage organs are low, having been used for spring growth. Management techniques timed to coincide with this reduction of stored carbohydrates in the target plant can reduce the potential for plant regrowth. Two annual low points in TNC storage have been determined for southern populations of Eurasian watermilfoil—in June and October (Madsen 1997b).

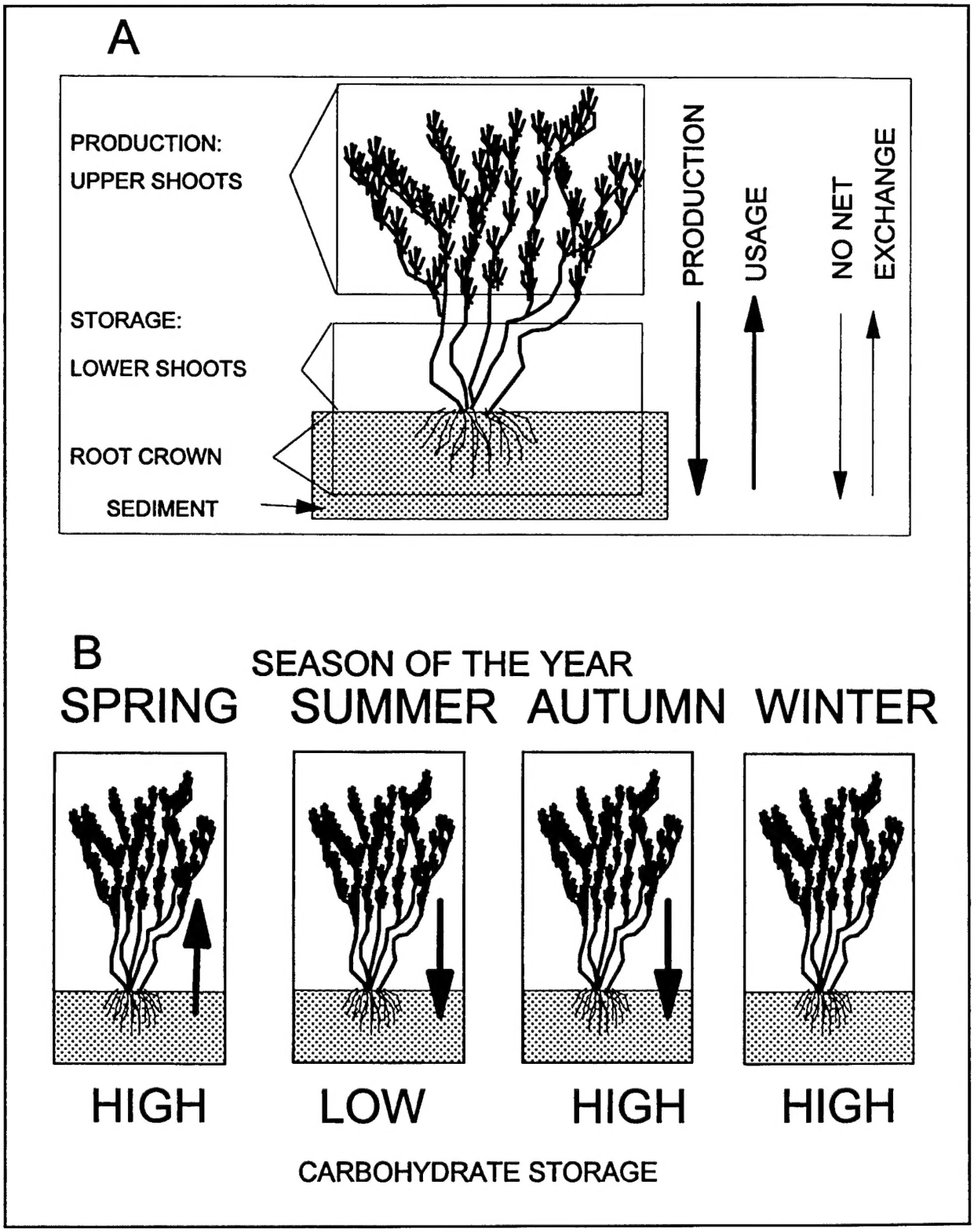

Figure 1. Diagrams of seasonal cycle of carbohydrate usage and storage in Eurasian watermilfoil (A) and of carbohydrate production and storage areas in a plant of Eurasian watermilfoil (B)

This study focused on the timing of herbicide application, to determine if treating during times of reduced TNC levels provides greater effectiveness.

METHODS: The study was conducted at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX (latitude 33°04'45"N, longitude 96°57'33"W) during the 1995 growing season. Two sprigs of Eurasian watermilfoil (15 cm) were planted in 3.75-L containers of Lewisville Lake pond sediment and placed into 1,125-L mesocosm tanks. The containers were planted in June 1994 prior to the herbicide application to permit adequate development of the plant and of TNC storage. The sediment was amended with a slow-release nutrient fertilizer briquette (14N, nitrogen—3P, phosphorus—3K, potassium) to provide sufficient nutrients for plant growth over the study period.

The primary low point of TNC storage in Eurasian watermilfoil (June) was bracketed by herbicide applications in May, June, and July (spring treatments 1-3). The secondary TNC low point (October), found only in southern Eurasian watermilfoil populations, was likewise bracketed by herbicide treatments in September, October, and November (fall 1-3). Each month's herbicide treatment was replicated in three tanks (1,125 L), with six untreated tanks used as references (experimental controls). Each treatment consisted of an exposure time of 48 hr of 3-ppm Aquathol-K. The tanks were flushed with pond water for 24 hr after the 48-hr exposure time.

Two harvests were conducted for each monthly treatment (Table 1). The first harvest was one week post-herbicide application, and the final was a common harvest—October 1995 for the spring cohort and May 1996 for the fall cohort. Each harvest consisted of removing three pots from each tank with three tanks per monthly treatment, providing nine samples per monthly treatment. In addition, nine containers were harvested from control tanks for reference.

All samples were separated into aboveground biomass (shoots) and belowground biomass (roots), dried at 60 °C for a minimum of 48 hr, then weighed. After obtaining a dry weight, samples were finely ground using a Cyclone Sampling Mill (UDY Corporation, Fort Collins, CO)

Table 1. Treatment Dates of Aquathol-K, and Dates of the First and Second Harvest for the Eurasian Watermilfoil Demonstration

Treatment and Date	First Harvest Date	Second Harvest Date
Spring 1 May 9, 1995	May 17, 1995	Oct 10, 1995
Spring 2 June 13, 1995	June 20, 1995	Oct 10, 1995
Spring 3 July 11, 1995	July 18, 1995	Oct 10, 1995
Fall 1 Sept 13, 1995	Sept 20, 1995	May 8, 1996
Fall 2 Oct 10, 1995	Oct 17, 1995	May 8, 1996
Fall 3 Nov 14, 1995	Nov 20, 1995	May 8, 1996

for TNC analysis (Swank and others 1982). Statistical analysis consisted of one-way analysis of variance and Tukey's comparison of the means (Zar 1984).

RESULTS AND DISCUSSION: Significant differences in biomass for shoots ($p < 0.01$) and roots ($p < 0.01$) were found between reference and the May treatment for the first post-treatment harvest (Figures 2a, 2b). No significant differences were detected between reference and treatment in biomass for June and July treatments when compared with untreated plants for the first post-treatment harvest. By the final harvest (October), Eurasian watermilfoil had not regrown following the June and July treatments (shoot $p < 0.01$, root $p < 0.01$) (Figures 2a, 2b).

The fall 1-week post-treatment harvest results indicated significant differences from the reference for shoot ($p < 0.03$) and root biomass ($p < 0.01$) for the October (the secondary low point) treatment (Figures 2c, 2d). No significant differences between reference and treatment were found for September or November for shoots and roots. Further, results obtained from the final post-treatment harvest (May) indicated reference shoot ($p < 0.01$) and root ($p < 0.01$) biomass to be significantly greater than the September and October biomass and the November root biomass. The November second post-treatment shoot biomass results were not significantly different from the reference (Figure 2c).

The TNC results are best explained by examining the root TNC concentrations. Within Eurasian watermilfoil plant, roots are the primary TNC storage organ during periods of stress (Madsen 1997b, Figure 1b). The concentrations of TNC in June, the primary low point as determined earlier (Madsen 1997b), were found to have the least stored concentrations of TNC in the roots for both the reference and the treatment of any spring treatment (Figure 3b). The May harvest TNC results indicated sufficient stored carbohydrates in the roots to withstand the herbicide treatment and to regrow, as evidenced by the second harvest biomass results. By October, no biomass was present from the June and July herbicide treatments, indicating a highly effective control strategy (Figure 2a).

The fall TNC results indicate the classic TNC storage pattern for Eurasian watermilfoil. During the early fall months, Eurasian watermilfoil began storage of TNC for overwintering. Significant differences were found for September and October harvests; however, by November (Figure 3d), the 1-week post-treatment results indicate that root TNC storage was high, therefore providing the plant with sufficient carbohydrate storage to regrow in the spring. This classic pattern was reflected in the second harvest results in May (Figures 2c, 2d), which found no significant difference in dry weight between the reference and the November post-treatment shoot biomass.

Initial TNC (percent dry weight) for shoot and root for all treatment dates can be seen in Figure 4b. In the initial May treatment harvest, root TNC was at approximately 13 percent, providing the Eurasian watermilfoil plants with sufficient carbohydrates to regrow, as seen in the final treatment shoot biomass for May. The June and July final shoot biomass results indicate insufficient stored TNC to regrow (Figure 4a). This is exemplified by June's initial root TNC (approximately 2.5 percent dry weight) while July had root TNC levels at 14 percent, similar to the May root TNC levels. This inconsistency in regrowth for July can possibly be explained as due to increased temperature levels during the summer months in Texas, which can negatively impact growth of Eurasian watermilfoil because of heat stress.

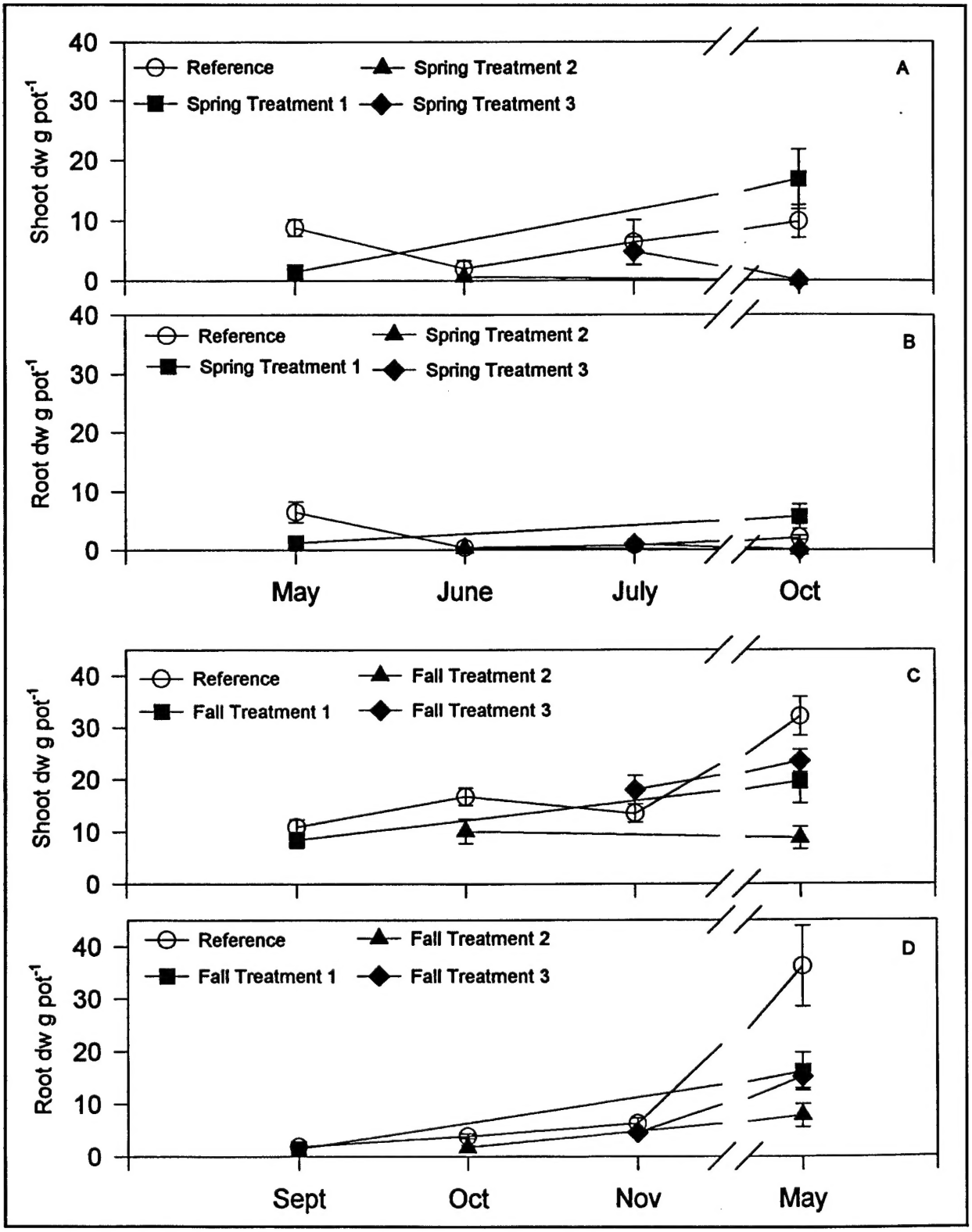

Figure 2. Eurasain watermilfoil biomass allocation for (A) shoot DW (dry weight, g pot^{-1}) for spring treatment, (B) root DW (g pot^{-1}) for spring treatment, (C) shoot DW (g pot^{-1}) for fall treatment, and (D) root DW (g pot^{-1}) for fall treatment. Bars indicate ±0.05 standard error of the mean

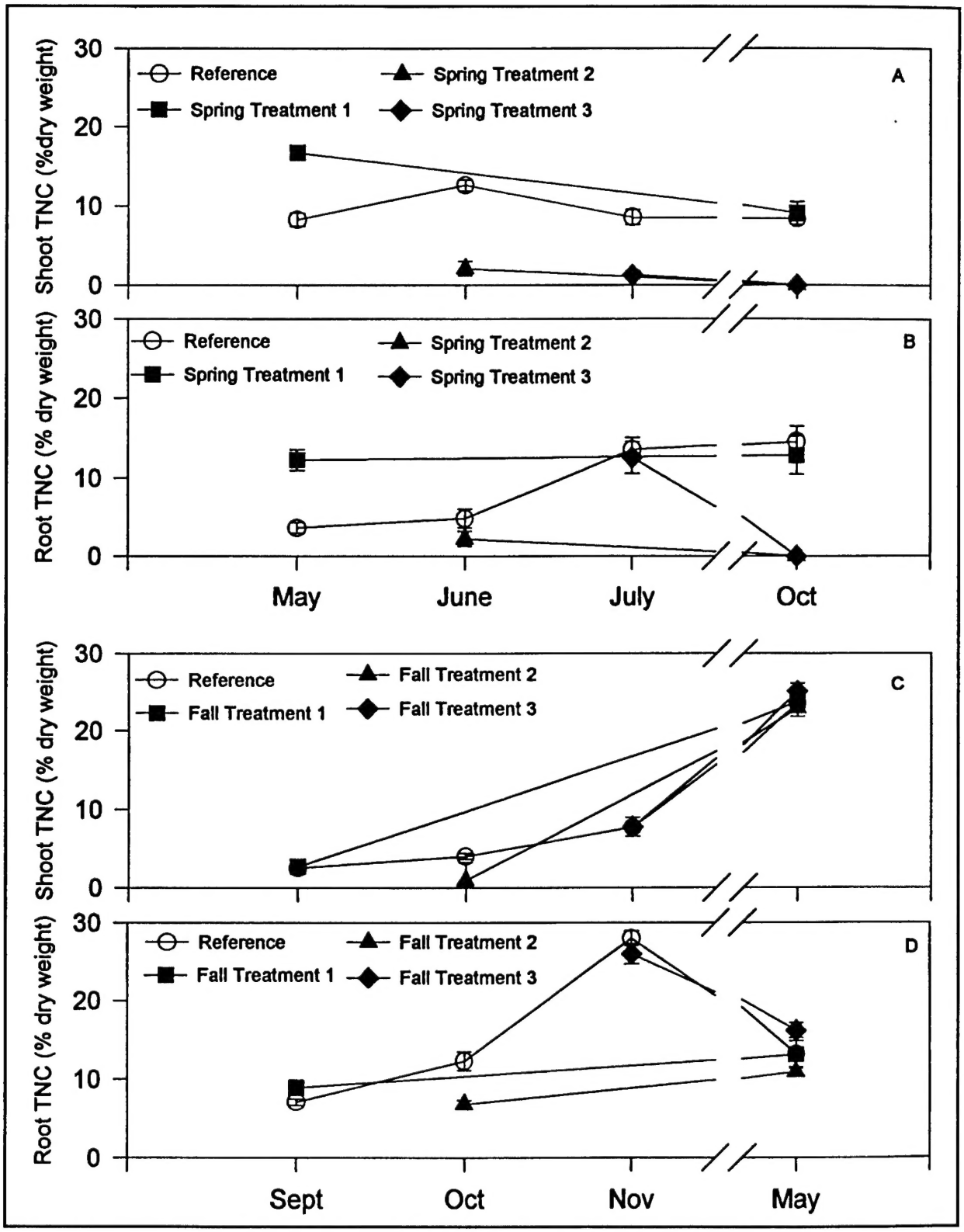

Figure 3. Total nonstructural carbohydrate concentrations as percent dry weight of Eurasian watermilfoil for (A) shoot TNC for spring treatments, (B) root TNC for spring treatments, (C) shoot TNC for fall treatments, and (D) root TNC for fall treatments. Bars indicate ±0.1 standard error of the mean

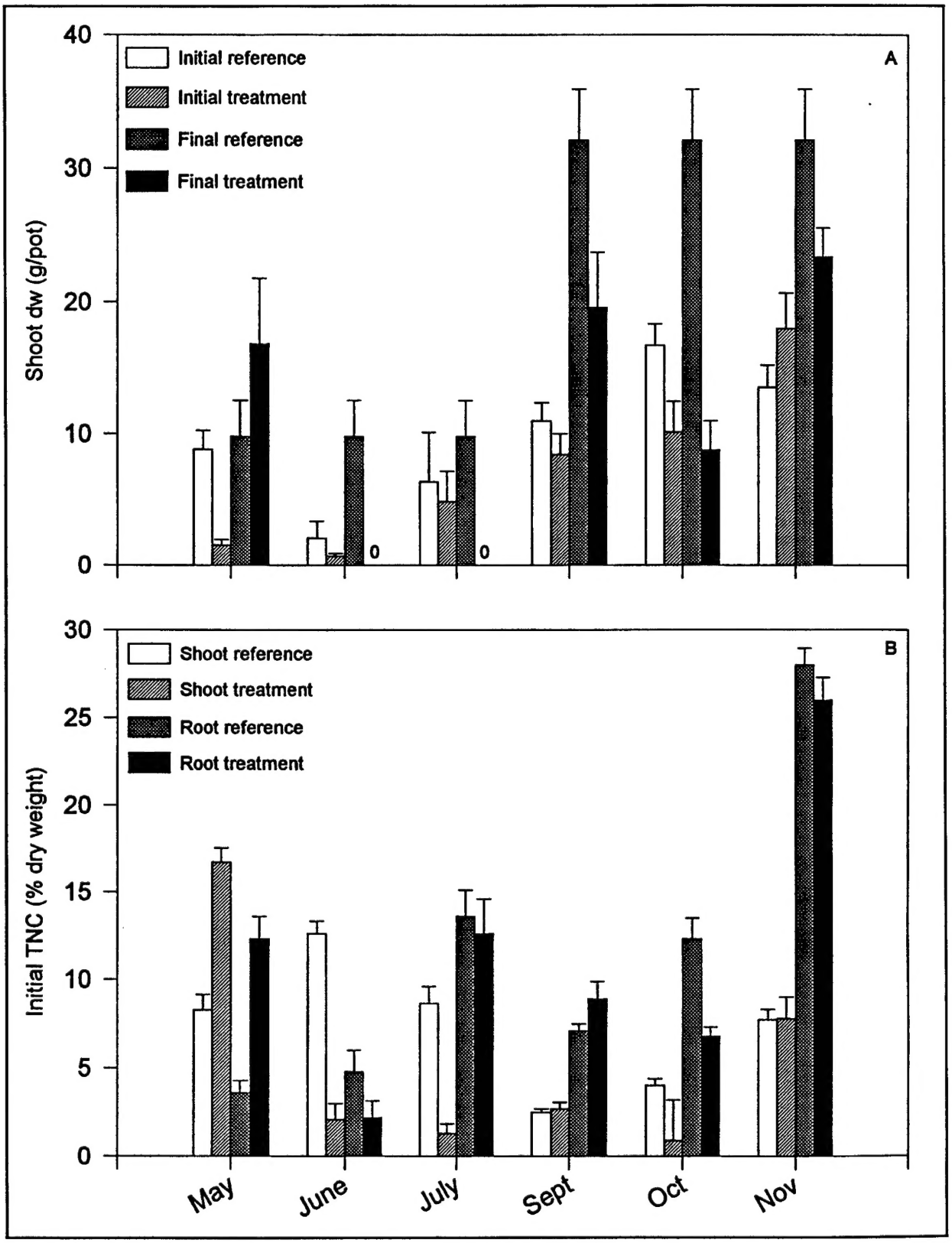

Figure 4. Eurasian watermilfoil biomass allocation for (A) initial and final biomass of the six treatments and reference and (B) initial TNC concentrations expressed as percent dry weight at time of treatment. Bars indicate ±0.1 standard error of the mean

During September and October, root TNC levels were at 9 percent (September) and 7 percent for October, respectively (Figure 4b). Although the plants recovered and regrew, there were still significant differences from the reference (Figure 4a). By November, however, the Eurasian watermilfoil plants had initiated TNC storage for overwintering. The root TNC levels were at 25 percent, thereby providing the treated and reference plants with sufficient carbohydrates for regrowth in the spring (Figures 4a, 4b).

Results obtained from this study indicate that synchronizing a herbicide application with the plant TNC storage levels can increase duration and efficacy of the herbicide application. Treatments applied during periods of highest Eurasian watermilfoil TNC concentration (May and November) had the highest recovery from the herbicide treatment, while herbicide treatments coinciding with reduced levels TNC were most effective with reduced levels of regrowth. This milfoil herbicide demonstration affirmed that low points in carbohydrate storage occur in summer (June and July) and early fall (October).

CONCLUSIONS: The effect of Aquathol-K application on Eurasian watermilfoil was studied to determine if chemical efficacy increases when timed to coincide with a low point of total nonstructural carbohydrates storage within Eurasian watermilfoil. Timing of any herbicide application is an important factor for overall treatment success. For this study, Aquathol-K was applied to Eurasian watermilfoil in both the spring and fall, bracketing the predetermined primary (June) and secondary (October) low points by 1 month before and after. The results indicated that shoot biomass had not regrown at the final harvest for June and July treatments, and TNC storage in roots was the lowest in June (primary low point) and July for both reference and treatment.

Shoot biomass was significantly reduced at the second post-treatment harvest for September and October compared with the control. However, the treatments were not as successful in retarding the shoot growth as when the herbicide was applied during the primary low point of TNC. The November shoot biomass was not significantly different from the reference. At this secondary low point, the plant contains more root TNC than at the primary low point, which allows the plant to regrow, although at a reduced rate.

These midsummer, midfall low points in TNC storage can vary depending on weather patterns and environmental conditions; however, they can be effectively used in an herbicide management program.

POINTS OF CONTACT: For additional information contact the authors, Ms. Chetta Owens (AScI Corporation, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX) and Dr. John Madsen, (601) 634-4631, *madsenj@mail.wes.army.mil*, or the manager of the Aquatic Plant Control Research Program, Dr. John W. Barko, (601) 634-3654, *barkoj@mail.wes.army.mil*. This technical note should be cited as:

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Integrated Fluridone-Fungal Pathogen Treatment of Four Submersed Plants

PURPOSE: This technical note describes an outdoor mesocosm investigation conducted to evaluate the efficacy and selectivity of the herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) and the fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (*Mt*), applied alone and in combination with one another, against hydrilla (*Hydrilla verticillata* (L.f.) Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L.), American pondweed (*Potamogeton nodosus* Poiret), and vallisneria (*Vallisneria americana* Michx.). Results of this research will determine the potential for integrating chemical and biological control tactics to improve the long-term management of nuisance aquatic weed species.

BACKGROUND: The goal of aquatic plant managers is to employ effective, cost-efficient, and environmentally compatible management strategies against nuisance and exotic weed species. Traditionally, these strategies have included the independent use of herbicides, biological organisms, mechanical harvesting, or habitat manipulation. Utilizing a multidisciplinary, integrated approach rather than applying a single control method may provide an alternate means for controlling nuisance plant infestations, and thus improve overall management efficiency.

The rationale for integrating control strategies is to combine the strengths of different technologies, thereby reducing inherent weaknesses of an individual technology when used alone. Integration of weed control practices has been successfully used in agro-ecosystems, but the concept has been limited in aquatic environments.

Several investigators have reported that the efficacy of some plant pathogens can be enhanced by integration with chemical herbicides (Charudattan 1986, Hoagland 1996, Netherland and Shearer 1996, Rayachhetry and Elliot 1997). In a recent review Hoagland (1996) stated that, although many pathogens have been characterized as bioherbicidal, most lack sufficient aggressiveness to overcome weed defense mechanisms to achieve adequate control. However, some herbicides and plant growth regulators can act to weaken natural plant defense systems, rendering them more susceptible to pathogen attack (Hoagland 1996).

Interactions between control agents may be antagonistic, synergistic, or additive, with additive and synergistic effects desirable for maximizing weed control. The potential advantages for implementing an integrated management strategy include increased efficacy, reduced herbicide and pathogen levels required for weed control, expanded pathogen host range, and a more economically and environmentally acceptable method of nuisance plant management (Charudattan 1986, Hoagland 1996).

Use of an integrated approach for managing the aquatic weeds waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and Eurasian watermilfoil has been investigated by others (Charudattan 1986; Sorsa, Nordheim, and Andrews 1988). Recently, Netherland and Shearer (1996) demonstrated that combining low doses of the systemic herbicide fluridone with a fungal pathogen, *Mt*,

was effective for controlling the nuisance exotic plant hydrilla in growth chamber trials. Applying a sublethal dose of fluridone (2 µg/L) with *Mt* at rates of 100 and 200 colony forming units (CFU)/ml reduced hydrilla biomass by >90 percent and was more efficacious than applying either control agent alone.

The integrated treatment provided the benefits of rapid biomass reduction exhibited by *Mt* and the long-term prevention of hydrilla regrowth provided by fluridone. In addition, integrated treatments reduced fluridone exposure requirements by approximately 50 days, which may broaden the use of this herbicide in aquatic environments where high water exchange has limited its use in the past. Fluridone generally requires a contact time of 60 to 90 days to achieve satisfactory hydrilla control and thus has limited use in aquatic systems where high water exchange precludes long chemical-plant exposure periods (Netherland, Getsinger, and Turner 1993; Netherland and Getsinger 1995).

Herbicide selectivity can often be achieved by applying lower than recommended dosages to sensitive vegetation. Selective removal of a nuisance plant species without damaging nontarget plants is a desirable goal for many aquatic plant management situations. One advantage that may result from integrating fluridone with *Mt* is that lowering the fluridone concentration may allow increased species selectivity.

Netherland, Getsinger, and Skogerboe (1997) demonstrated in a mesocosm study that 60- and 90-day exposures of 5 µg/L fluridone were sufficient to significantly reduce Eurasian watermilfoil biomass with no effect on biomass production of nontarget species (elodea (*Elodea canadensis* Mich.), American pondweed, sago pondweed (*Potamogeton pectinatus* L.), and vallisneria), whereas higher fluridone rates (10 to 20 µg/L) injured all nontarget species. Thus, the potential exists to control the growth of noxious species with reduced rates of fluridone, without affecting desirable native species.

The objectives of this study were to verify laboratory efficacy of integrating fluridone with *Mt* for control of hydrilla, the target weed, under outdoor growing conditions and to determine the selectivity of fluridone-*Mt* treatment on other submersed plant species.

MATERIALS AND METHODS: This study was conducted in an outdoor mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, which consists of large tanks (1.4 m tall by 2.6 m in diameter) that hold approximately 6,500 L of water. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. Further description of this mesocosm system can be found in Dick, Getsinger, and Smart (1997).

For this study, each of the 18 mesocosm tanks was divided into four equal sections, with netting to accommodate each of the four plant species. The netting allowed water flow between the divided areas but restricted plant growth to each section. Plants were grown in plastic pots (19.7 cm tall by 19.7 cm in diameter) filled with nutrient-enriched soil (one Woodace briquette (14-3-3) plus 10 g ammonium sulfate per pot). Nine pots of each plant species (three plants per pot) were placed in each tank section. Hydrilla (dioecious biotype) and Eurasian watermilfoil

were propagated from 10-cm apical cuttings and planted 4 to 5 cm into the soil. American pondweed and vallisneria were initiated from pregerminated tubers placed 4 to 5 cm into the soil.

All plants and tubers used in this study were collected from pond-grown cultures at the LAERF. Plants were allowed to establish in the mesocosm tanks for 2 months prior to herbicide-pathogen treatment. At the time of treatment, hydrilla and Eurasian watermilfoil had grown to the water surface, American pondweed had formed a surface canopy of floating leaves, and vallisneria was well established.

Treatments were applied on June 19, 1996, and included 5 µg/L fluridone, 100 and 200 CFU/ml of *Mt*, integrated treatments of 5 µg/L fluridone + 100 or 200 CFU/ml *Mt*, and untreated controls. Fluridone stock solutions were prepared from the liquid commercial formulation Sonar AS (479 g active ingredient per liter). *Mt* (isolated from hydrilla in Texas) was applied as a thick slurry of live fungal mycelium. The *Mt* inoculum was prepared as described by Shearer (1996). Both fluridone and *Mt* were applied by pouring the chemical solution and the mycelial suspension evenly over the water surface. Integrated treatments were applied simultaneously to designated tanks.

Plant biomass was harvested at 21, 42, and 84 days after treatment (DAT). At each harvest, three randomly selected pots of each plant species were removed from each mesocosm tank. Aboveground biomass was clipped at the sediment surface, washed to remove algae and debris, and dried to a constant weight at 60 °C. Plant biomass was recorded as grams dry weight per pot.

Fresh tissue samples (four samples per plant species per tank) were collected pretreatment and at each post-treatment harvest for chlorophyll analysis. The tissue selected for this procedure varied for each plant species and included 4-cm stem apices of hydrilla and Eurasian watermilfoil, floating leaves of American pondweed, and 4-cm leaf segments of vallisneria. Total chlorophyll (a and b) was measured using a DMSO extraction procedure (Hiscox and Israelstam 1979).

Water samples were collected from all fluridone-treated tanks (at 1, 2, 3, and 7 DAT, weekly thereafter through 42 DAT, and at 63 and 84 DAT) to confirm initial fluridone treatment rates and to determine herbicide dissipation. Samples were collected in 500-ml amber polyethylene bottles and frozen until analysis. Fluridone residues were detected using a high performance liquid chromatography (HPLC) procedure.[1] Residue data were subjected to linear regression procedures, and the results obtained were used to determine the half-life of fluridone under these experimental conditions.

Treatments were randomly assigned to mesocosm tanks and were replicated three times. At each sampling interval, biomass and chlorophyll data were subjected to analysis of variance and treatment means compared using Fisher's protected Least Significant Difference (LSD) test at the 0.05 level of significance.

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1 Lilly Research Laboratory. (1980). "Method AM-AA-CA-R005-AC-755: Determination of fluridone in water by direct injection high pressure liquid chromatography," Eli Lilly and Company, Greenfield, IN.

RESULTS AND DISCUSSION: Residue analyses at 1 day after treatment (data not shown) showed that the initial target fluridone concentration (5 µg/L) was achieved in all chemically treated mesocosm tanks. Subsequent water residue data were used to determine fluridone dissipation over time. Regression analysis established that under these experimental conditions, the average half-life of fluridone in herbicide-treated tanks was 49 days (Figure 1). Fluridone dissipation was comparable to dissipation rates reported by Netherland, Getsinger, and Skogerboe (1997) under similar experimental conditions.

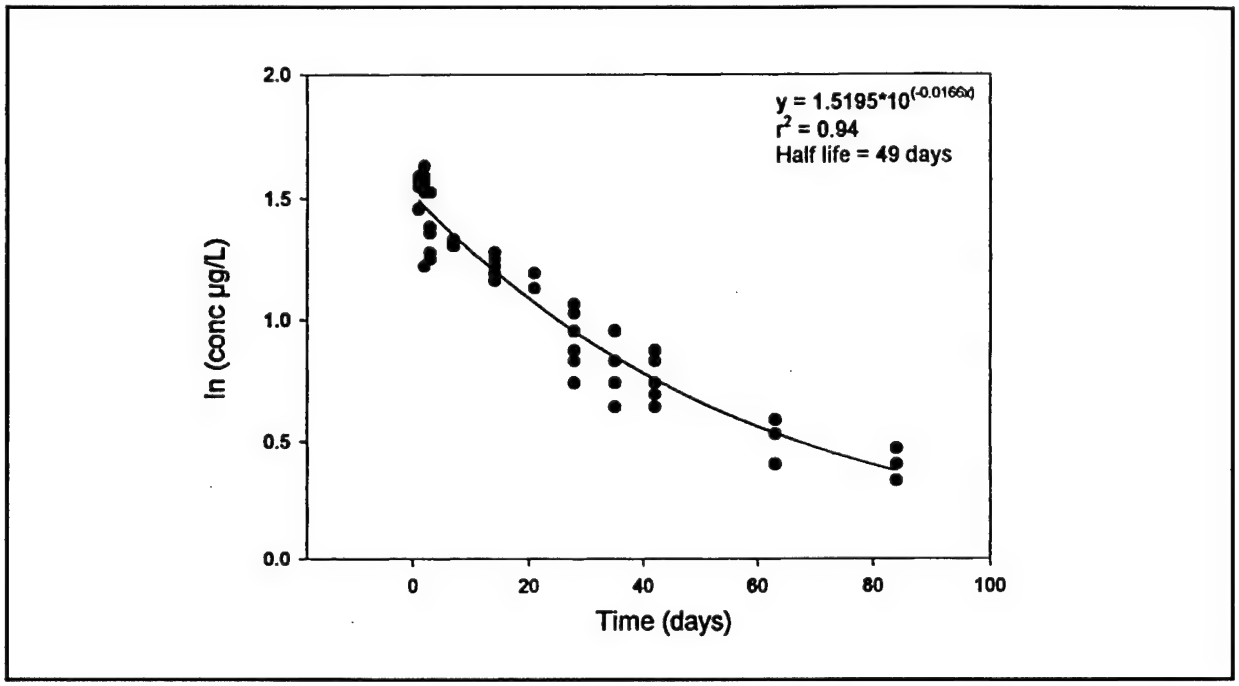

Figure 1. Dissipation of fluridone in water collected from large outdoor mesocosm tanks at Lewisville, TX. Initial treatment rate was 5 µg L^{-1}

Treatment effects on dry weight biomass varied greatly among plant species (Figure 2). The greatest response was observed on the target plant, hydrilla (Figure 2a). At 21 DAT, treatment with either fluridone alone or 200 CFU/ml *Mt* was sufficient to reduce hydrilla biomass by an average of 36 percent. However, the combined application of *Mt* plus fluridone reduced biomass up to 75 percent compared with untreated plants. By 84 DAT, the combined treatments resulted in a 93 percent reduction in hydrilla biomass. Both fluridone alone and 200 CFU/ml *Mt* reduced hydrilla biomass by 40 percent at the final harvest. Statistically, there were no differences between the two rates of *Mt* or between fluridone alone and *Mt* at 200 CFU/ml on hydrilla.

Characteristic injury symptoms of fluridone and *Mt* were observed on hydrilla. Successful fungal infection was noted on all *Mt*-treated tanks 10 DAT and was identified by leaf tip chlorosis and stem defoliation. Although biomass was not significantly different between the two rates of *Mt*, disease symptoms were visibly more abundant on tanks treated with the higher than the lower rate of *Mt*. At the first post-treatment harvest, new and healthy hydrilla growth (lateral shoots from viable stems) also was present in all tanks treated with *Mt* by itself. Fluridone effects on hydrilla (pink stem coloration and bleached leaves on new tissues) were observed 21 DAT.

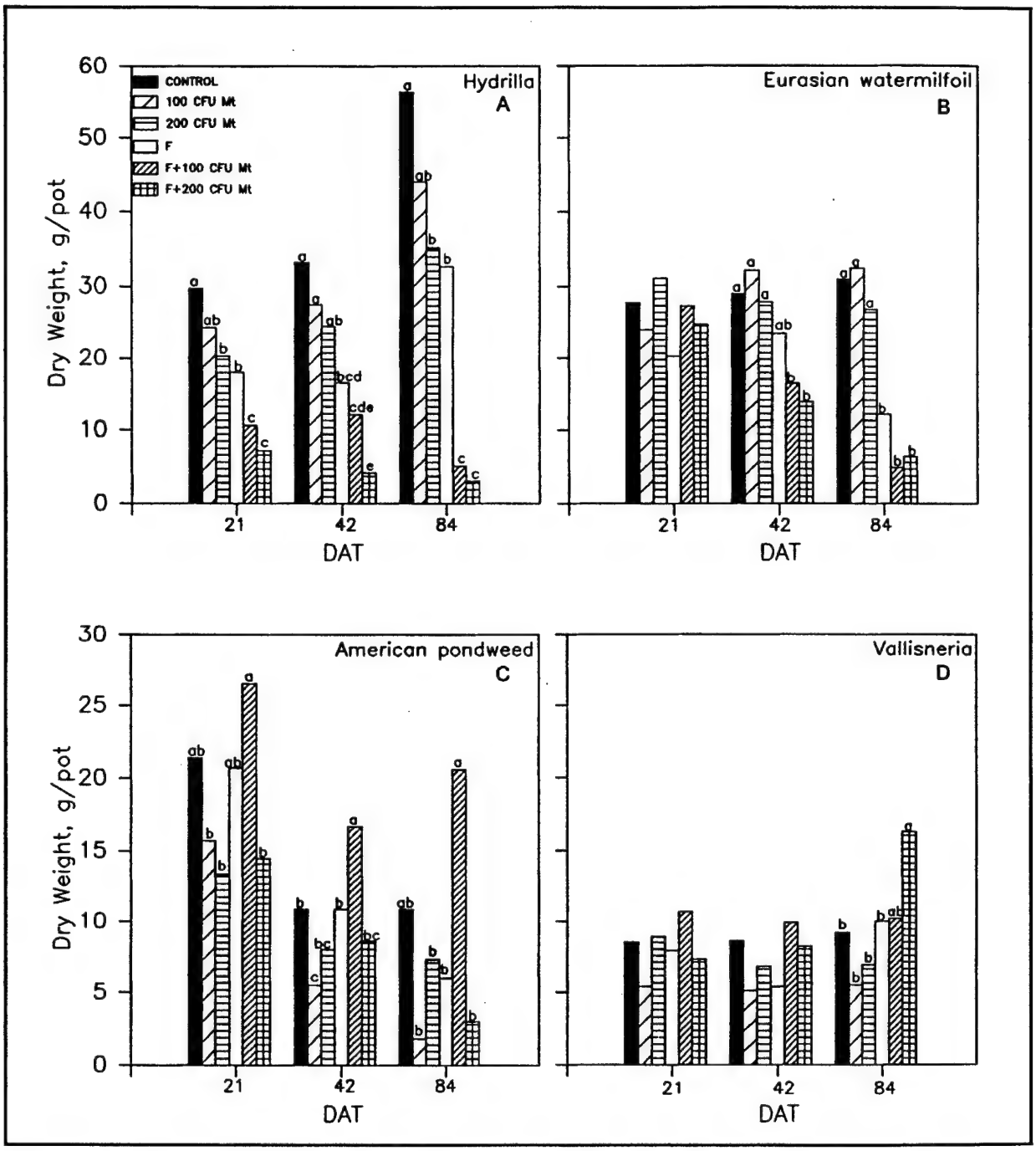

Figure 2. Mean dry weight biomass of hydrilla (A), Eurasian watermilfoil (B), American pondweed (C), and vallisneria (D) at 21, 42, and 84 days after treatment (DAT) following application of *Mt* at 100 and 200 colony forming units (CFU) per milliliter, fluridone (F = 5 µg/L fluridone), and integrated treatments of fluridone + *Mt*. Within each sample time, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's protected LSD test

Fluridone, but not *Mt*, symptomology was also observed on Eurasian watermilfoil. Neither vallisneria nor American pondweed displayed visible symptoms of fungal infection or fluridone leaf bleaching.

Although Eurasian watermilfoil was not the target plant in this study, treatment with fluridone alone and fluridone plus either 100 or 200 CFU/ml *Mt* reduced Eurasian watermilfoil biomass by 75 percent at 84 DAT (Figure 2b). Unlike the synergistic effect observed on hydrilla, the response on Eurasian watermilfoil was likely due to fluridone itself, as there were no statistical differences between treatments of fluridone alone and those integrated with *Mt*. The fact that effects on biomass were not observed until late in the study (42 DAT) further implies fluridone activity as the main source of efficacy.

Fluridone is a slow-acting herbicide compared to the quick infection response observed with *Mt* (Netherland and Shearer 1996). Results are consistent with other outdoor mesocosm studies in which fluridone at a rate of 5 µg/L was sufficient to reduce Eurasian watermilfoil biomass (Netherland, Getsinger, and Skogerboe 1997). Strains of *Mt* (other than that used in this study) have been isolated for activity on Eurasian watermilfoil and were found to be effective in greenhouse trials (Gunner and others 1990). Combining milfoil-specific strains of *Mt* with fluridone may have potential as an integrated approach for management of Eurasian watermilfoil, and should be evaluated.

Nontarget species were less affected by fluridone and *Mt*. Compared with untreated plants, none of the treatments inhibited biomass of American pondweed at 21 DAT (Figure 2c). Results were variable at subsequent harvests. For example, *Mt* at 100 CFU/ml significantly reduced biomass by 50 percent 42 DAT, while treatment with fluridone + 100 CFU/ml *Mt* resulted in a significant increase (35 percent) in biomass. By the end of the study, none of the treatments was statistically different from controls; however, fluridone + 100 CFU/ml *Mt* showed significantly higher biomass when compared with other fluridone or *Mt* treatments.

Some of the observed variation in biomass data can be attributed to insect damage. At 21 DAT, floating leaves of American pondweed had been severely decimated by an unidentified species of whitefly (*Trialeurodes* sp.) and a common aquatic insect identified as the larva of the waterlily leafcutter (*Synclita obliteralis* (Walker)). Infestation was not evenly distributed among tanks (some tanks were not infested at all) and may account for the variability in biomass data observed on this plant species. American pondweed in two of the three replicate tanks treated with fluridone + 100 CFU/ml *Mt* did not show insect damage, which may explain the high biomass levels recorded for this treatment.

Vallisneria biomass was not inhibited by any of the applied treatments (Figure 2d). No statistical differences among treatments were noted at 21 and 42 DAT, and by the final harvest, only fluridone + 200 CFU/ml *Mt* was significantly different from untreated plants. For reasons unknown, this treatment showed a 44 percent increase in biomass compared with untreated plants.

With the exception of American pondweed, all treatments that included fluridone significantly reduced total chlorophyll content in sampled tissues (Table 1). Hydrilla was most sensitive, with chlorophyll decreases of >70 percent measured at 21 DAT and a >50 percent decrease recorded

thereafter. For Eurasian watermilfoil, chlorophyll content in fluridone-treated plants was 32 to 39 percent less than that of untreated plants throughout the study. Initially, vallisneria showed reduced leaf chlorophyll (by 29 percent at 21 DAT). However, at 84 DAT there were no differences among treatments, indicating plant recovery. For all plant species, *Mt* alone did not affect total chlorophyll at the times sampled. Netherland and Shearer (1996) showed reduced chlorophyll content in hydrilla at 7 and 14 DAT with 100 and 200 CFU/ml *Mt*, but the effects dissipated by 28 DAT.

Table 1. Effect of Fluridone, *Mt*, and Fluridone + *Mt* Treatments on Total Chlorophyll Content of Four Submersed Plant Species

Species	Treatment (µg/L + CFU)[1]	Total Chlorophyll Content (mg g-1 fr wt)			
			Days after Treatment[2]		
		Pretreatment	21 DAT	42 DAT	84 DAT
Hydrilla	Untreated	1.17	1.11 a	1.09 a	1.12 a
	0 + 100	1.04	0.95 a	1.15 a	1.14 a
	0 + 200	1.02	0.97 a	1.03 a	1.22 a
	5 + 0	1.21	0.20 c	0.50 b	0.44 b
	5 + 100	1.14	0.30 bc	0.44 b	0.54 b
	5 + 200	1.16	0.39 b	0.52 b	0.56 b
	(LSD)	NS	(0.19)	(0.25)	(0.23)
E. watermilfoil	Untreated	1.44	1.56 a	1.73 a	1.35 a
	0 + 100	1.58	1.53 a	1.70 a	1.51 a
	0 + 200	1.47	1.65 a	1.77 a	1.49 a
	5 + 0	1.40	1.05 b	1.03 b	0.98 b
	5 + 100	1.45	1.09 b	1.00 b	0.81 b
	5 + 200	1.50	1.06 b	1.14 b	0.92 b
	(LSD)	NS	(0.25)	(0.20)	(0.26)
American pondweed	Untreated	1.42	1.10	1.40	1.43 b
	0 + 100	1.53	0.86	1.30	1.41 b
	0 + 200	1.74	0.97	1.40	1.51 b
	5 + 0	1.54	1.19	1.39	1.32 b
	5 + 100	1.70	1.11	1.30	1.27 b
	5 + 200	1.63	0.95	1.15	1.83
	(LSD)	NS	NS	NS	(0.32)
Vallisneria	Untreated	0.86	0.78 b	0.85 ab	0.68
	0 + 100	0.86	0.97 a	0.78 abc	1.35
	0 + 200	0.87	0.78 b	0.93 a	0.78
	5 + 0	0.87	0.52 c	0.66 bc	0.50
	5 + 100	0.92	0.62 c	0.64 bc	0.46
	5 + 200	0.74	0.51 c	0.58 c	0.63
	(LSD)	NS	(0.13)	(0.22)	NS

Note: Within columns, means followed by different letters are significantly different (Least Significant Difference, $P \leq 0.05$); NS = not significant.
[1] Fluridone concentration (expressed in µg/L) plus colony forming units of *Mycoleptodiscus terrestris*.

The results of this study confirm those observed in growth chamber studies by Netherland and Shearer (1996). For hydrilla, a beneficial synergistic interaction was observed with combined applications of 5 µg/L fluridone with either 100 or 200 CFU/ml *Mt*. Neither control agent alone provided adequate hydrilla control. For Eurasian watermilfoil, 5 µg/L fluridone was sufficient to significantly reduce biomass, which was consistent with reports that maintenance of low doses of fluridone over time can significantly inhibit biomass production (Netherland, Getsinger, and Skogerboe 1997). There was no advantage to integrating fluridone with *Mt* on Eurasian watermilfoil. At the rates applied, the strain of *Mt* utilized in this study was ineffective on

Eurasian watermilfoil. Other strains of *Mt* have been isolated for pathogenicity on this plant species and may be potential candidates for integrating with fluridone.

The desired level of selectivity was achieved with the integrated treatments applied in this study. Biomass of American pondweed and vallisneria was not severely impacted by treatment rates sufficient to control the target species, hydrilla. The results demonstrated that by integrating fluridone and *Mt*, a low herbicide rate that reduced the likelihood of chemical damage to nontarget species could be used. The potential for selectivity gives further merit to the concept of integrated weed management.

FUTURE WORK: Future research will focus on larger scale field testing of fluridone-*Mt* treatments for controlling hydrilla, as well as evaluating other potential herbicide-pathogen combinations for aquatic plant management. Development of a granular *Mt* formulation to provide an easier and more uniform means of application has also been initiated.

Initial field tests were conducted in June 1997 in nine small ponds located at the Center for Aquatic Plants in Gainesville, FL. These ponds (0.15 acre-foot) were nearly 100 percent covered with hydrilla and represent situations where fluridone injury is often delayed due to the lack of active plant growth in a dense canopy of hydrilla. Treatments included fluridone alone (15 µg/L), *Mt* alone (150 CFU/ml), fluridone + *Mt* (15 µg/L + 150 CFU/ml), and fluridone plus the contact herbicide copper (15 µg/L + 250 µg/L). Hydrilla biomass and chlorophyll content as well as water quality changes were monitored at 0, 6, and 12 weeks after treatment. Results of this study and additional pond studies, to be conducted at the Lewisville Aquatic Ecosystem Research Facility, will be discussed in future technical notes.

POINTS OF CONTACT: For additional information contact the authors, Ms. Linda S. Nelson, (601) 634-2656, *nelsonl@mail.wes.army.mil,* Dr. Judy F. Shearer, (601) 634-2516, *shearej@mail.wes.army.mil,* and Mr. Michael D. Netherland, (601) 634-3889, *netherml@mail.wes.army.mil*, or the manager of the Aquatic Plant Control Research Program, Dr. John W. Barko, (601) 634-3654, *barkoj@mail.wes.army.mil.* This technical note should be cited as follows:

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Littoral Fishes Response, Upper Lake Marion, SC, Following Triploid Grass Carp Hydrilla Control

PURPOSE: This technical note summarizes a 7-year study that was conducted to investigate the effects of hydrilla control by triploid grass carp on fishes in upper Lake Marion, South Carolina.

BACKGROUND: Hydrilla *(Hydrilla verticillata)* became established in upper Lake Marion during the early 1980s and, by 1988, had colonized over 4,000 hectares. In 1989, triploid grass carp (*Ctenopharyngodon idella*) were stocked into upper Lake Marion to control hydrilla. By 1994, almost 600,000 fish had been released into the Santee Cooper system (Lakes Marion and Moultrie and the connecting canal). Extensive surface coverage of hydrilla persisted through 1991, began to decline in 1992, and was reduced to less than 60 hectares in upper Lake Marion by 1994.

As part of the study reported herein, fish in upper Lake Marion were sampled for 7 years to evaluate the effects of decreasing hydrilla coverage on fish abundance. A boat-mounted electroshocker was used to quantify relative abundance and species composition of fishes at 10 permanent locations distributed throughout the upper lake (Figure 1).

FISH COMMUNITY: A total of 16,306 fish representing 64 species were collected in the 176 (15-min) electroshocking samples. The taxonomically dominant family was Centrarchidae (sunfishes), comprising 15 species and accounting for 22 percent of the total number of fish collected. The numerically dominant family was Clupeidae (shad), comprising 5 species and accounting for 37 percent of the total number of fish collected. Other common families included Cyprinidae (minnows) and Catostomidae (suckers).

Dominant species (≥4 percent), in decreasing order of abundance, were threadfin shad (*Dorosoma petenense*), golden shiner (*Notemigonus crysoleucas*), gizzard shad (*Dorosoma cepedianum*), largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), eastern silvery minnow (*Hybognathus regius*), blueback herring (*Alosa aestivalis*), redear sunfish (*Lepomis microlophus*), and inland silverside (*Menidia beryllina*).

COMPARISON OF FISH ABUNDANCE BETWEEN HIGH AND LOW HYDRILLA COVERAGE: The number of species collected during high (1989-1992) and low (1993-1994) hydrilla coverage was similar (51 and 50 species, respectively). However, mean catch of all species combined significantly increased during low hydrilla coverage (Figure 2). Littoral and pelagic fishes showed similar results. Frequently collected littoral species (>0.8 percent of total catch) that increased significantly after hydrilla declined included bowfin (*Amia calva*), golden shiner, lake chubsucker (*Erimyzon sucetta*), bluegill, redear sunfish, largemouth bass, and yellow perch (*Perca flavescens*) (Figure 3). Mean catch of coastal shiner (*Notropis petersoni*) and black-spotted sunfish (*Lepomis punctatus*) also increased significantly during low hydrilla

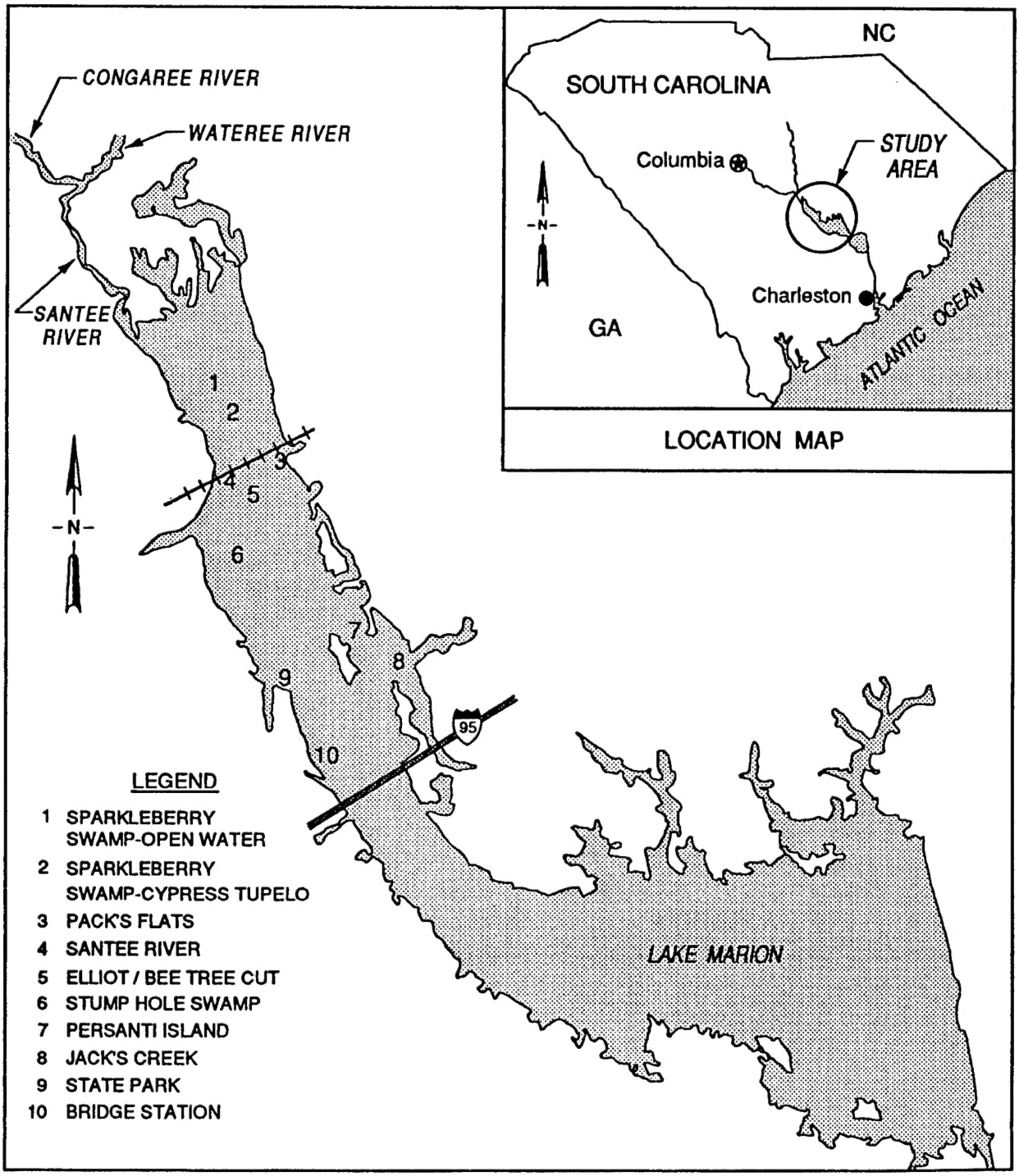

Figure 1. Study area sampling locations, upper Lake Marion, South Carolina

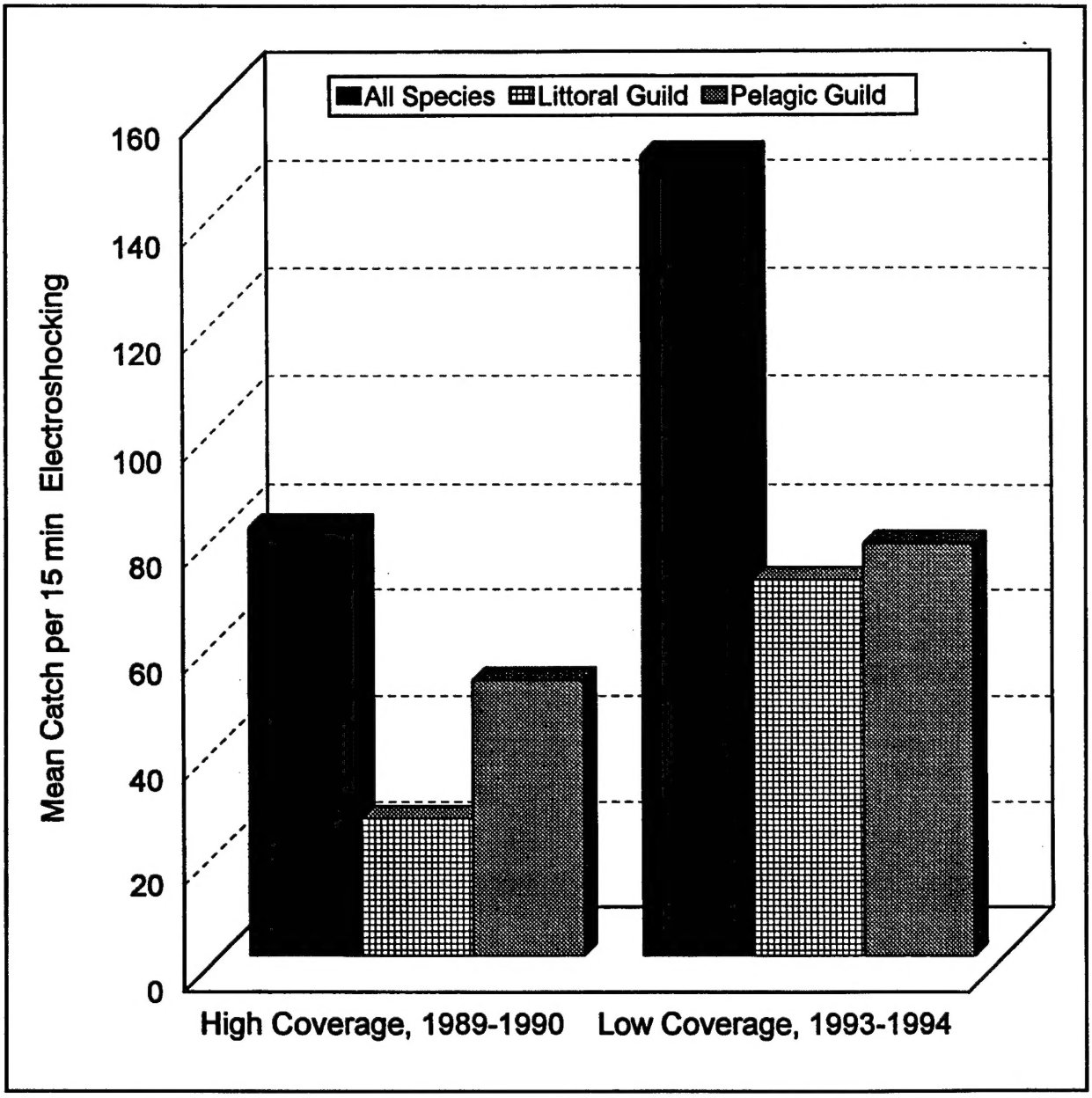

Figure 2. Fish abundance, for all species combined and by guild, during periods of high versus low hydrilla coverage

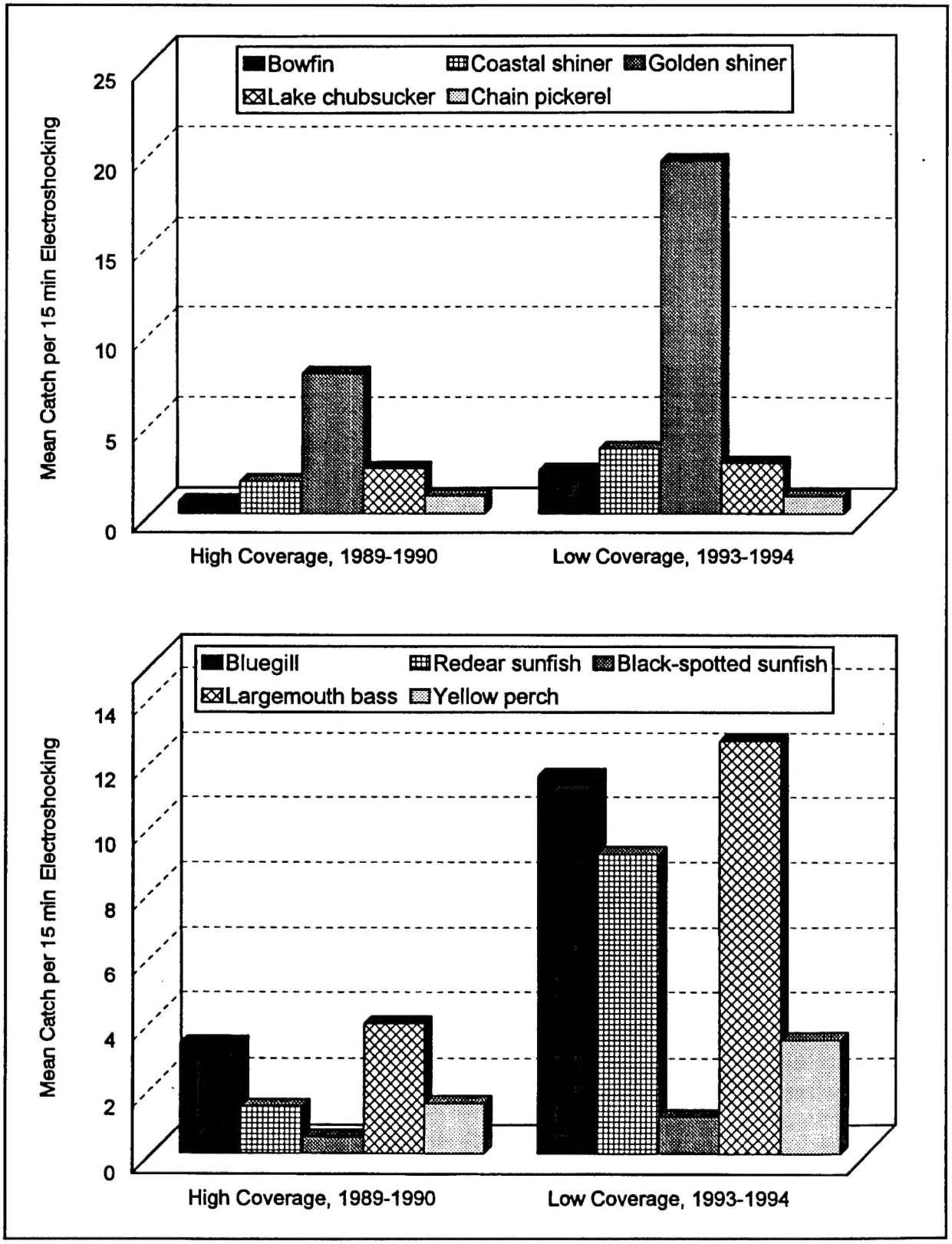

Figure 3. Fish abundance, for individual species, during periods of high versus low hydrilla coverage

coverage, but to a lesser degree. There was no significant difference in mean catch of chain pickerel (*E. niger*) between the two time periods.

CONCLUSIONS: Grass carp reduced the surface coverage of hydrilla in upper Lake Marion from approximately 50 percent to less than 10 percent, while abundant structure near the shoreline (in the form of standing timber, subsurface submersed vegetation, and floating and emergent species) remained. Thus, the underwater landscape of upper Lake Marion was shifted from monospecific stands of hydrilla to intermediate levels of structural complexity. Consequently, grass carp effectively controlled hydrilla and resulted in no detectable negative effects on the fish assemblage during the study.

POINTS OF CONTACT: For additional information contact the authors, Dr. K. Jack Killgore, (601) 634-3397, *killgok@mail.wes.army.mil*, and Dr. J. P. Kirk, (601) 634-3060, *kirkj@mail.wes.army.mil*, or the manager of the Aquatic Plant Control Research Program, Dr. John W. Barko, (601) 634-3654, *barkoj@mail.wes.army.mil*. This technical note should be cited as follows:

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